Am. J. Hum. Genet. 64:788–792, 1999

Autosomal Dominant Myopathy with Proximal Weakness and Early Respiratory Muscle Involvement Maps to Chromosome 2q

Piero Nicolao,[1,3] Fengqing Xiang,[1,2] Lars-Gunnar Gunnarsson,[4] Bruno Giometto,[3] Lars Edström,[2] Maria Anvret,[1,2] and Zhiping Zhang[1]

Departments of [1]Molecular Medicine and [2]Clinical Neuroscience, Karolinska Hospital, Stockholm; [3]Department of Neurology and Psychiatry (Second Neurological Clinic), University of Padova, Italy; and [4]Department of Neurology and Neurophysiology, Örebro Medical Central Hospital, Örebro, Sweden

Summary

Two Swedish families with autosomal dominant myopathy, who also had proximal weakness, early respiratory failure, and characteristic cytoplasmic bodies in the affected muscle biopsies, were screened for linkage by means of the human genome screening set (Cooperative Human Linkage Center Human Screening Set/Weber version 6). Most chromosome regions were completely excluded by linkage analysis (LOD score <-2). Linkage to the chromosomal region 2q24-q31 was established. A maximum combined two-point LOD score of 4.87 at a recombination fraction of 0 was obtained with marker *D2S1245*. Haplotype analysis indicated that the gene responsible for the disease is likely to be located in the 17-cM region between markers *D2S2384* and *D2S364*. The affected individuals from these two families share an identical haplotype, which suggests a common origin.

Introduction

In 1990 Edström et al. described 16 individuals, from seven families, who had autosomal dominant, adult-onset myopathy with early respiratory muscle involvement. All patients were characterized by proximal weakness of the upper and lower extremities. In many patients the respiratory muscles, especially the diaphragm, were involved. Neck flexors were typically affected, and foot extensor weakness was also present in some patients. The age at onset of significant symptoms varied from the second to the fifth decades, with an average age of ~35 years. Creatine kinase (CK) levels were normal or slightly elevated in all cases. Typical muscle biopsy samples showed, at the light microscopic level, cytoplasmic bodies that were highly positive for rhodamine-conjugated phalloidin. Rhodamine-conjugated phalloidin selectively binds to F-actin. At the ultrastructural level, these bodies were seen to be composed of thin filaments and dense material related to the Z-discs.

Received July 10, 1998; accepted for publication January 6, 1999; electronically published February 17, 1999.

Address for correspondence and reprints: Dr. Zhiping Zhang, Department of Molecular Medicine, Clinical Neurogenetic Unit, CMM-L8-02-058, Karolinska Hospital, S-17176, Stockholm, Sweden. E-mail: zhiping.zhang@cmm.ki.se

0002-9297/99/6403-0016$02.00

Subjects and Methods

Genomic DNA was extracted from the peripheral blood leukocytes of 8 affected and 17 healthy individuals from two of the seven families described by Edström et al. (1990) (fig. 1). All individuals were examined at the Department of Neurology, Örebro Hospital, and most were seen by L. Edström and/or L.-G. Gunnarsson. Most patients underwent a muscle biopsy. A normal dystrophin and α-sarcoglycan expression pattern was seen in the muscle tissue. Most patients fulfilled the diagnostic criteria for autosomal dominant limb-girdle muscular dystrophy (LGMD) (Bushby 1995): onset of the disease in the pelvic or shoulder girdle muscles, inevitable and slow progression of symptoms, normal to mildly raised serum CK activity, and myopathic changes on electromyography (EMG) and on muscle biopsy. Because muscle necrosis and fibrosis were not evident in the muscle biopsy samples from affected patients, the disorder described by Edström et al. (1990) was defined as an autosomal dominant myopathy and not as an LGMD. The study protocol was accepted by the ethical board at Örebro Hospital (1995-05-15).

Genotype Analysis

Three hundred ninety-one microsatellite markers from the Cooperative Human Linkage Center (CHLC) Human Screening Set/Weber version 6 were used for the initial screening. Additional markers from Research Genetics were applied. Genotypes were determined by PCR amplification using ^{33}P-labeled nucleotides. PCR reac-

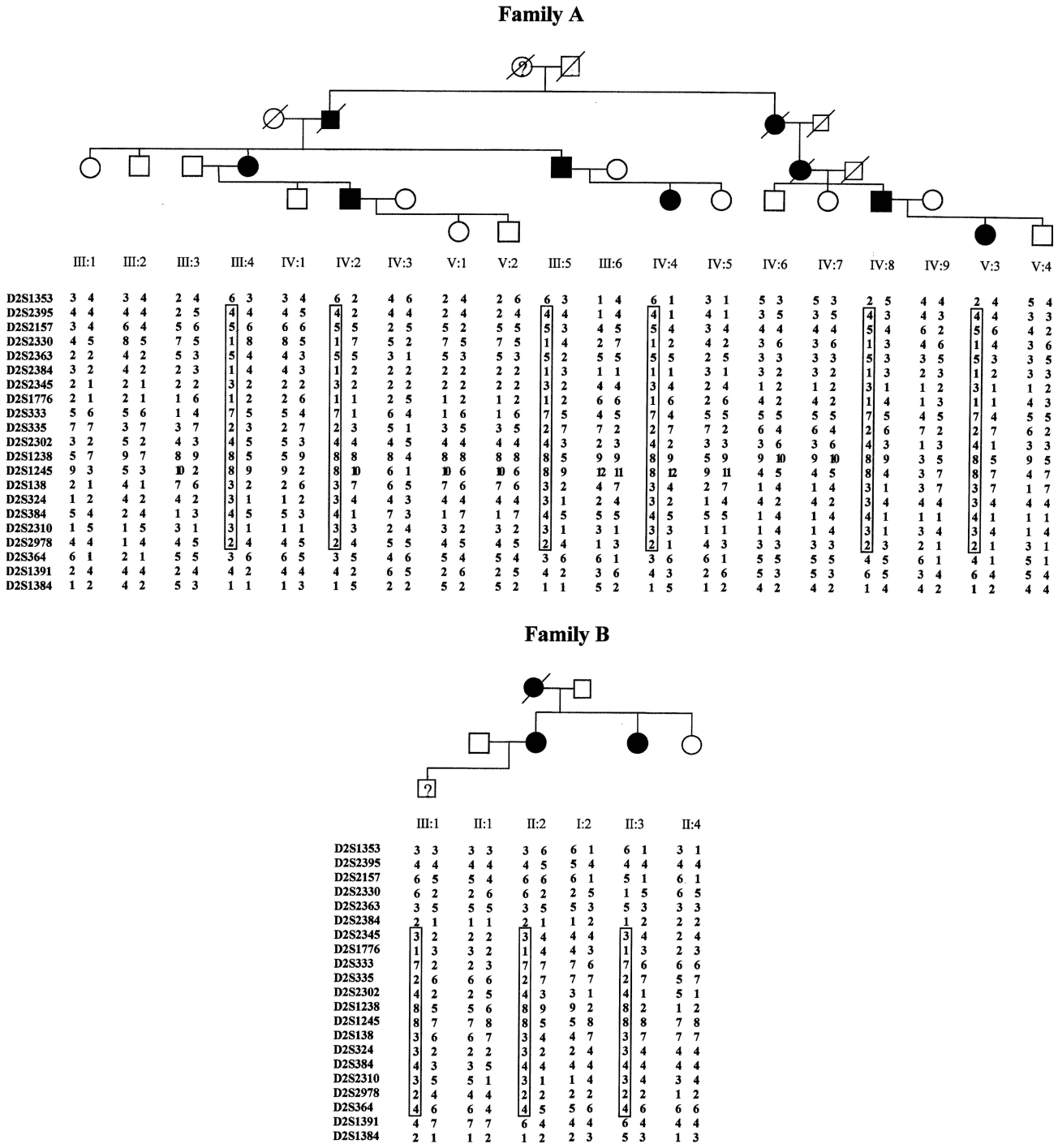

Figure 1 Haplotypes of two Swedish families (A and B) who had autosomal dominant myopathy, with markers from *D2S1353* to *D2S1384*. Haplotypes were constructed assuming a minimum number of recombinations. The haplotype segregating with the disease is boxed. A question mark (?) indicates unknown phenotype.

tions were performed on 96-well microtiter plates in 10-μl reaction volumes containing 50 ng of genomic DNA, 2 pmol of each primer, and 0.5 U of *Taq* polymerase (Sigma). The conditions for PCR were 95°C for 1 min, 55°C–60°C for 1 min, and 72°C for 1 min, with a final extension step of 72°C for 7 min. The PCR products were separated on 6% denaturing polyacrylamide gels. The alleles were visualized by autoradiography.

Two-Point Linkage Analysis

Two-point LOD scores were calculated by using the MLINK program, as implemented in the FASTLINK package, version 3.0P (Lathrop and Lalouel 1984; Cottingham et al. 1993; Terwilliger and Ott 1994). The order and the approximate distances of the markers were obtained from the Généthon integrated map and the Marshfield Medical Research Foundation's Chromosome 2 Sex-Averaged linkage map. The frequency of the disease gene in the population was assumed to be 1:10,000, with an autosomal dominant inheritance pattern and 100% penetrance. Allele frequencies and male/female recombination fractions were assumed to be equal. Haplotypes were constructed by assuming a minimum number of recombinations. In the linkage analysis, because of the late onset of the symptoms, the disease status of individuals aged <25 years was coded as unknown (individual III:1 in family B).

Results

A whole-genome screen, using the CHLC Human Screening Set/Weber version 6, was performed for the two Swedish families with this autosomal dominant myopathy. The screening set consists of 391 markers with an average distance interval of 10 cM. Because of the clinical similarity between the present myopathy and the autosomal dominant LGMD, haplotype analysis was performed for candidate regions of LGMD1A, 1B, and 1C (5q22-q31, 1q11-q21, and 3p25). The possibility that the described disease could represent an allelic form of these LGMDs was completely excluded. Except for the region of 2q24-q31, most chromosome regions were excluded by linkage and haplotype analysis (LOD score < -2). A positive LOD score (2.29 at recombination fraction [θ] of zero) was obtained in family A with marker *D2S1776,* located on chromosome 2q24. Additional highly informative markers close to *D2S1776* were used to confirm or exclude the linkage. Linkage to this region was established. A combined maximum LOD score of 4.87 was obtained at $\theta = 0$ with marker *D2S1245* (table 1). This is the highest LOD score attainable in these two families (A and B).

All affected individuals from family A share a common haplotype that spans the region from *D2S2395* to *D2S2978*. In family B, the region shared by the affected individuals extends from *D2S2345* to *D2S1391* (fig. 1). Loci that are proximal to *D2S2384* are excluded by a recombination event in individual II:2 from family B. Exclusion of loci that are distal to *D2S364* is based on a recombination event in family A that has resulted in the presence of different haplotypes in the affected individuals: III:4, III:5, IV:2, and IV:4. All affected individuals from these two families share an identical haplotype from *D2S2345* to *D2S2978*, suggesting a common origin (results obtained with the most informative markers are summarized in table 1).

Table 1

Combined Pairwise LOD Scores between the Disease Gene and Markers of Chromosome 2q24-q31

Marker	Genetic Distance[a]	LOD Score at θ =						
		.00	.001	.005	.010	.020	.30	.40
D2S1353	2	−∞	.28	1.36	1.55	1.29	.73	.19
D2S2395	2.9	1.61	1.60	1.51	1.39	1.09	.75	.37
D2S2157	0	−∞	1.93	2.33	2.25	1.73	1.04	.37
D2S2330	0	−∞	2.83	3.19	3.06	2.43	1.59	.67
D2S2363	0	2.80	2.75	2.54	2.26	1.65	1.0	.36
D2S2384	1.6	3.82	3.74	3.45	3.07	2.23	1.33	.47
D2S2345	2	4.47	4.38	4.01	3.54	2.54	1.47	.50
D2S1776	2.9	2.89	2.83	2.58	2.27	1.63	.97	.34
D2S333	0	4.22	4.14	3.79	3.34	2.38	1.37	.40
D2S335	0	4.26	4.18	3.85	3.42	2.49	1.51	.50
D2S2302	1.6	4.07	3.99	3.66	3.23	2.31	1.34	.41
D2S1238	5	4.20	4.11	3.78	3.34	2.42	1.42	.46
D2S1245	.4	4.87	4.78	4.41	3.93	2.89	1.78	.67
D2S138	1.1	4.64	4.55	4.18	3.71	2.68	1.59	.57
D2S324	1.1	4.52	4.43	4.06	3.59	2.58	1.52	.52
D2S384	0	1.63	1.62	1.53	1.41	1.13	.79	.40
D2S2310	0	3.05	3.00	2.79	2.48	1.80	1.03	.28
D2S2978	1.1	4.75	4.66	4.29	3.82	2.80	1.71	.66
D2S364	0	.45	2.46	2.81	2.65	1.99	1.16	.40
D2S1391	14.2	−∞	−1.06	.15	.50	.54	.31	.08
D2S1384		−∞	1.25	1.71	1.70	1.34	.82	.32

[a] Estimated distance from the next marker in centimorgans according to the Marshfield Medical Research Foundation's Chromosome 2 Sex-Averaged linkage map.

Discussion

Respiratory muscle involvement is a common occurrence in the late stages of many muscular dystrophies, such as Duchenne muscular dystrophy and myotonic dystrophy. Disorders with early respiratory involvement include, together with our myopathy, acid maltase deficiency in adults (MIM232300) (Rosenow and Engel 1978) and nemaline myopathy (NEM2; MIM 256030) (Dubowitz 1978). Many patients in the families investigated required assisted ventilation a few years after the onset of symptoms. A peculiar characteristic of the patients described by Edström et al. (1990) was the presence of cytoplasmic bodies in the muscle fibers. These bodies stain with rhodamine-conjugated phalloidin and, at the ultrastructural level, appear to be composed of filamentous material.

Tibial muscular dystrophy (TMD; MIM 600334), a relatively rare autosomal dominant myopathy, has recently been mapped to chromosome 2q31 between the markers *D2S2188* and *D2S2310* (Haravuori et al. 1998). This locus is completely included in our mapping region, and the two diseases have some features in com-

mon, such as autosomal dominant inheritance, late onset, and normal to slightly elevated serum CK levels. Some patients described by Edström et al. (1990) had early involvement of dorsal foot extensors. Additional clinical and histological findings reveal that the two diseases are, in fact, different. In TMD the weakness is usually limited to the tibial anterior muscles, and the myopathic changes evident in the muscle biopsy samples are unspecific (Udd et al. 1992, 1993). Similarly, LGMD2B (MIM 253601) and two distal myopathies—Miyoshi myopathy (MM; MIM 254130) and Welander distal myopathy (MIM 160500)—have been mapped to the same region of chromosome 2p12-p14 (Bashir et al. 1994, 1996; Bejaoui et al. 1995, 1998; Åhlberg G, Borg K, Edström L, Anvret M, unpublished data). LGMD2B and Miyoshi myopathy are both caused by mutations of the *Dysferlin* gene (MIM 603009) (Bashir et al. 1998; Liu et al. 1998). This raises the possibility that the disorder described by Edström et al. (1990) and TMD might represent allelic variants of the same gene.

The autosomal recessive form of nemaline myopathy has been mapped by linkage analysis to a 13-cM region of chromosome 2q21.2-q22 between the markers *D2S150* and *D2S142* (Wallgren-Pettersson et al. 1995). This region is ~8 cM proximal to our region and contains the *Nebulin* gene (MIM 161650). *Nebulin* is considered to be a functional candidate gene for nemaline myopathy (Labeit and Kolmerer 1995*a*; Pelin et al. 1997). Recessive nemaline myopathy and the present disease share some clinical and pathological findings. Tissue from the muscle biopsy samples from patients with nemaline myopathy, however, shows a regular lattice pattern by electron microscopy that characterizes the rod bodies (Fardeau 1982). In addition, the region of 2q21.2-q22 is excluded for our patients by linkage and haplotype analysis (LOD score < -2 at $\theta = 0$ for the markers *D2S1792, D2S1399,* and *D2S2299*, which, according to the sex-averaged linkage map of the Marshfield Medical Research Foundation, map within the recessive nemaline myopathy locus).

The present disease is located on a 17-cM region on chromosome 2q24-q31. Several candidate genes, among which are some specifically expressed in the muscle, map within this area. The *Titin* gene (MIM 188840) is considered to be a functional candidate for our myopathy. Titin, or connectin, is the largest polypeptide known and forms, together with nebulin, the third filament system of the striated muscle fibers. Titin molecules form filaments >1μm long (Nave et al. 1989; Suzuki et al. 1994), which span from the Z-discs to the M-lines (Fürst et al. 1988). This filament system accounts for the passive tension of the resting muscle and contributes to the assembly of striated muscle myofibrils (Labeit and Kolmerer 1995*b*). The whole coding region of the gene (~300 kb) maps within our candidate region, between the markers *D2S335* and *D2S364* (Pelin et al. 1997).

Acknowledgments

This work was supported by grants from the Swedish Medical Research Council (3875), the Petrus and Augusta Hedlund Foundation, the Söderberg Foundation, and the "Association for rare diseases Mauro Baschirotto." We are grateful to Dr. Jill Thyboll, for collecting the samples, and to Ann-Christin Thelander, for her excellent technical assistance.

Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

Généthon, http://www.genethon.fr (for order and distances of markers)

Center for Medical Genetics, Marshfield Medical Research Foundation, http://www.marshmed.org/genetics/ (for order and distances of markers)

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for acid maltase deficiency [MIM 232300], NEM2 [MIM 256030], TMD [MIM 600334], LGMD2B [MIM 253601], MM [MIM 254130], Welander distal myopathy [MIM 160500], Dysferlin [MIM 603009], Nebulin [MIM 161650], and Titin [MIM 188840])

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